

Supplementary Material

Whole genome sequencing details

Whole-genome libraries for NGS were prepared with the Nextera XT kit (Illumina, San Diego, CA, USA), and sequencing was performed with 2x250-bp paired-end reads on the Illumina MiSeq. Quality control of NGS sequencing run was performed by the Bioinformatic Unit using FastQC v0.11.8 and Trimmomatic v.0.33.

We generated a *C. diphtheriae* and *C. ulcerans* cg-MLST scheme, defining specific target loci for whole-genome sequencing data typing, by using the default options of the SeqSphere+ target definer tool (Ridom, Munster, Germany). As reference we used the genome of strain NC 13129 from the National Center for Biotechnology Information (NCBI) (accession number no. BX248353.1/NC_002935.2) for *C. diphtheria* and the genome of strain NC 017317.1 for *C. ulcerans*. We included all 23 complete *C. diphtheriae* available from NCBI as query sequences (accession nos. NC_016782.1, NC_016783.1, NC_016785.1, NC_016786.1, NC_016787.1, NC_016788.1, NC_016789.1, NC_016790.1, NC_016799.1, NC_016800.1, NC_016801.1, NC_016802.1, NZ_CP018331.1, NZ_CP020410.2, NZ_CP025209.1, NZ_CP029644.1, LT_990688.1, NZ_LR134538.1, NZ_LR1345537.1, NZ_CP039523.1, CP038789.1, NZ_CP039522.1, and NZ_CP039523.1) and 16 complete *C. ulcerans* available from NCBI as query sequences (accession nos. NC_015683.1, NC_0108101.1, NZ_CP009500.1, NZ_CP009583.1, NZ_CP009622.1, NZ_CP009716.1, NZ_CP011095.1, NZ_CP011913.1, NZ_CP010818.1, NZ_CP021417.1, NZ_LT906443.1, NZ_LS483400.1, NZ_LS483416.1, NZ_AP019662.1, NZ_AP019663.1, and NZ_CP046863.1). The complete genome of strain NC_CPO25209.1 was used to exclude horizontally transferred genes to prevent their inclusion in the cgMLST typing scheme.

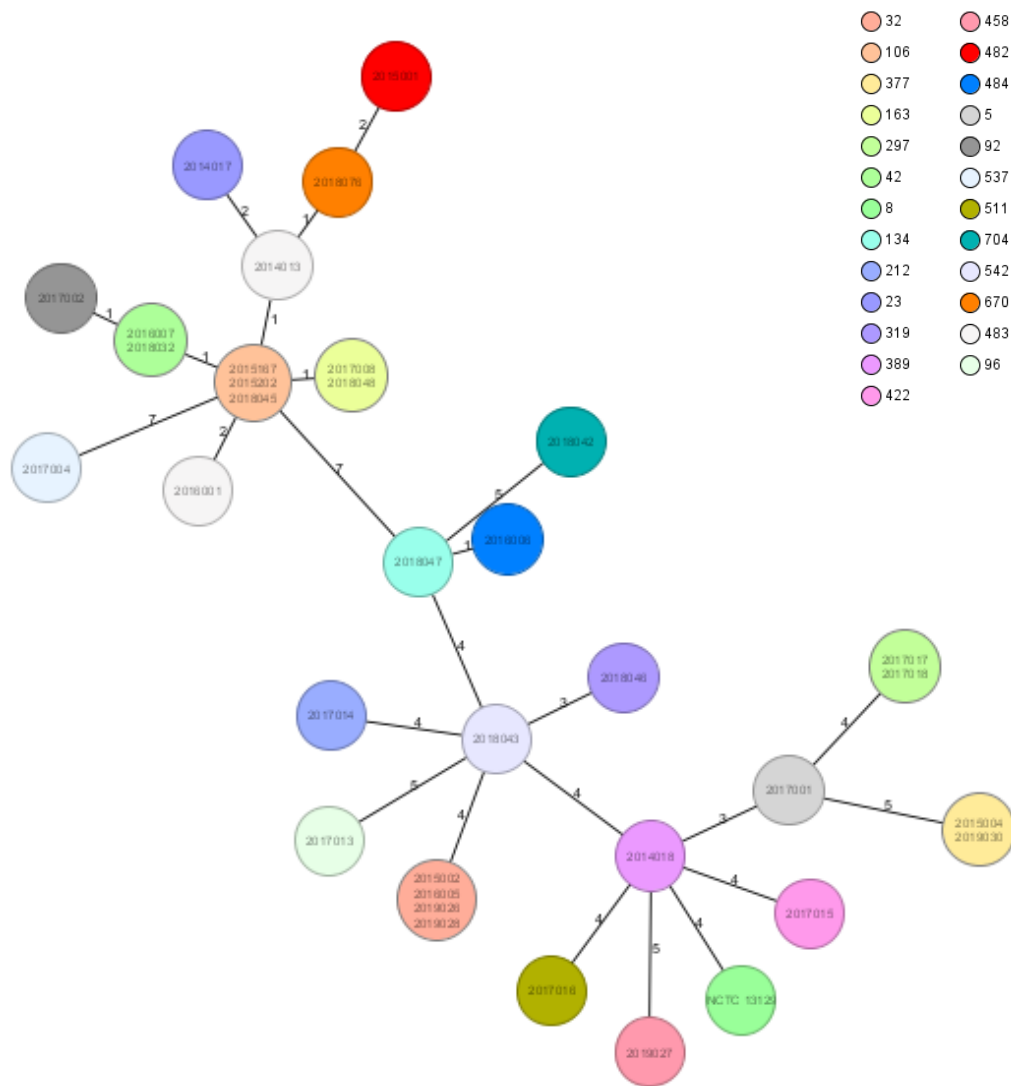


Fig. S1 - Minimum spanning tree of the clonal analysis of the *C. diphtheriae* isolates generated using the eBURST algorithm. Isolates color coded according to the eBURST group in which they were allocated on pubMLST.

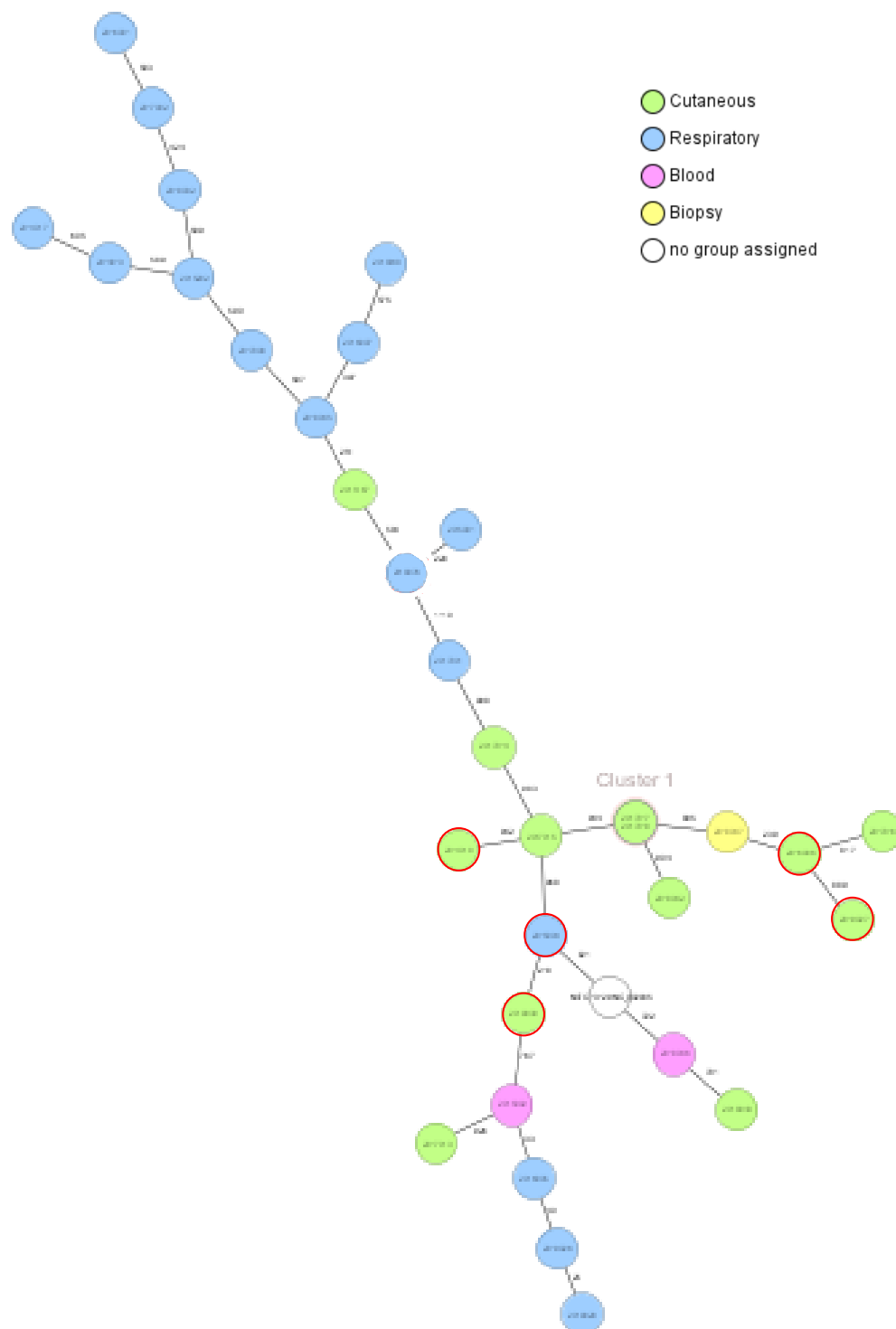


Fig. S2 - Minimum spanning tree of *C. diphtheriae* isolates generated using a cluster distance threshold of ≤ 5 alleles. Colour coded according to the site of infection as indicated and all toxigenic isolates are marked with a solid red circle. The reference genome NC 13129 is white.

In silico antimicrobial resistance determinants

ResFinder analysis identified a total of 38 resistance genes with two or more resistance genes in 26% (10/39) of the isolates, all of which were identified in the *C. diphtheriae* isolates. Three of the toxigenic isolates harbored resistance determinants; one harbored aminoglycoside resistance genes (*aadA1/aph(3'')-Ib/aph(3'')-Ia/aph(6)-id*), chloramphenicol resistance genes (*cmx/cmlA1*), a sulphonamide resistance gene (*sul1*) and a trimethoprim resistance gene (*dfrA15*). The second toxigenic isolate harbored aminoglycoside resistance genes (*aph(3'')-Ib/aph(3'')-Ia/aph(6)-id*), a chloramphenicol resistance gene (*cmx*), a sulphonamide resistance gene (*sul1*) as well as the tetracycline resistance gene *tet(33)*. The third toxigenic isolate harboring resistance genes to aminoglycosides (*aadA5*), a sulphonamide resistance gene (*sul1*) as well as a trimethoprim resistance gene (*dfrA1*). 13% (5/39) of the isolates harbored at least one aminoglycoside resistance gene, 8% (3/39) harbor at least one chloramphenicol resistance gene, 26% (10/39) harbour the sulphonamide resistance gene *sul1*, 18% (7/39) harbour a tetracycline resistance gene and 13% (5/39) harbored a trimethoprim resistance gene.

Table S1 – NGA-based phylogeny of diphtheria-related pathogenicity factors in different *Corynebacterium* spp. implies species-specific virulence transmission

| isolate information | | tox status wetlab | | mobile element details | | | GC content | | detected prophage regions (PHASTER results) | | | | | | | | | | detected PAI region coordinates | | | | | | | |
|---------------------|----------------|-------------------|----------|--------------------------------|--------------------------------|-------------|---------------------|-----------------|---|-----------------------------|--------------------------|----------------|-------------------------------------|---|---|---|--|--|--|---|---|--|--|---|-----------------------------------|--|
| sample name | species | tox PCR | Elk | phage/PAI tox gene surrounding | length phage / PAI region (kb) | region type | genome assembly GC% | phage / PAI GC% | prophage completeness | prophage completeness score | prophage #Total Proteins | prophage_start | prophage_end | prophage region: most common phage | prophage results: URL | start of tRNA-Arg (LacI) 6 kb 5' of tox | start of phage integrase similar to AKN76028 | start of alternative phage integrase similar to AKN76371 | start of hyp. prot. with tRNA + DNA binding motif, similar to AKN76029 | start of hyp. prot. similar to AKN76030 | start of hyp. prot. similar to AKN76032 | start of hyp. prot. with tRNA + DNA binding motif, similar to AKN76033 | start of hyp. prot. with tRNA + DNA binding motif, similar to AKN76035 | start of putative transposase similar to AKN76035 | start of 100bp tRNA-Period Repeat | |
| 2014013 | C. belfanti | negative | na | no | 6.7 | prophage | 53.8% | 55.1% | incomplete | 10 | 8 | 38.787 | 45.556 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_43302458 | na | na | na | na | na | na | na | na | na | | |
| 2014013 | C. belfanti | negative | na | no | 10.9 | prophage | 53.8% | 56.0% | incomplete | 10 | 15 | 35.872 | 46.837 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_43302468 | na | na | na | na | na | na | na | na | na | | |
| 2014013 | C. belfanti | negative | na | no | 6.3 | prophage | 53.8% | 59.0% | incomplete | 20 | 7 | 1.799 | 10.167 | PHAGE_Gordon_Blueberry_NC_030943(2) | https://phaster.ca/biocommons/22_43302468 | na | na | na | na | na | na | na | na | na | | |
| 2014013 | C. belfanti | negative | na | no | 7.3 | prophage | 53.8% | 53.7% | incomplete | 10 | 13 | 1 | 7.308 | PHAGE_Coryne_Foushouh_NC_042139(7) | https://phaster.ca/biocommons/22_43302468 | na | na | na | na | na | na | na | na | na | | |
| 2014017 | C. belfanti | negative | na | no | 10.9 | prophage | 53.8% | 56.0% | incomplete | 10 | 15 | 273 | 11.239 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_20225a66 | na | na | na | na | na | na | na | na | na | | |
| 2014017 | C. belfanti | negative | na | no | 6.8 | prophage | 53.8% | 55.0% | incomplete | 10 | 8 | 28.805 | 35.647 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_20225a66 | na | na | na | na | na | na | na | na | na | | |
| 2014017 | C. belfanti | negative | na | no | 8.8 | prophage | 53.8% | 53.4% | incomplete | 20 | 6 | 49.340 | 58.178 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_20225a66 | na | na | na | na | na | na | na | na | na | | |
| 2014017 | C. belfanti | negative | na | no | 8.5 | prophage | 53.8% | 52.4% | incomplete | 30 | 11 | 43.741 | 52.337 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_20225a66 | na | na | na | na | na | na | na | na | na | | |
| 2015001 | C. belfanti | negative | na | no | 7.6 | prophage | 53.7% | 53.1% | incomplete | 20 | 14 | 33.968 | 41.572 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_40842a66 | na | na | na | na | na | na | na | na | na | | |
| 2015001 | C. belfanti | negative | na | no | 10.1 | prophage | 53.7% | 56.0% | incomplete | 10 | 21 | 44.841 | 54.977 | PHAGE_Mycobacterium_Gardann_NC_01124(3) | https://phaster.ca/biocommons/22_40842a66 | na | na | na | na | na | na | na | na | na | | |
| 2015001 | C. belfanti | negative | na | no | 24.2 | prophage | 53.7% | 54.4% | incomplete | 10 | 32 | 2 | 24.282 | PHAGE_Coryne_Foushouh_NC_042139(8) | https://phaster.ca/biocommons/22_40842a66 | na | na | na | na | na | na | na | na | na | | |
| 2015001 | C. belfanti | negative | na | no | 15.3 | prophage | 53.7% | 52.5% | incomplete | 10 | 25 | 714 | 16.087 | PHAGE_Gordon_Neyrac_NC_010040(8) | https://phaster.ca/biocommons/22_40842a66 | na | na | na | na | na | na | na | na | na | | |
| 2015167 | C. belfanti | negative | na | no | 10.9 | prophage | 53.7% | 56.0% | incomplete | 10 | 15 | 78.147 | 89.111 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_431808-02 | na | na | na | na | na | na | na | na | na | | |
| 2015167 | C. belfanti | negative | na | no | 6.7 | prophage | 53.7% | 55.1% | incomplete | 10 | 9 | 38.794 | 45.563 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_431808-02 | na | na | na | na | na | na | na | na | na | | |
| 2015202 | C. belfanti | negative | na | no | 7.5 | prophage | 53.9% | 53.7% | incomplete | 20 | 14 | 32.397 | 39.958 | PHAGE_Coryne_Foushouh_NC_042139(2) | https://phaster.ca/biocommons/22_82423999 | na | na | na | na | na | na | na | na | na | | |
| 2016001 | C. belfanti | negative | na | no | 18.3 | prophage | 53.8% | 56.4% | questionable | 70 | 18 | 47.600 | 65.911 | PHAGE_Gordon_GMAI_NC_030902(6) | https://phaster.ca/biocommons/22_43155b10 | na | na | na | na | na | na | na | na | na | | |
| 2016007 | C. belfanti | negative | na | no | 6.8 | prophage | 53.7% | 54.9% | incomplete | 10 | 8 | 73.848 | 80.690 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_43041928 | na | na | na | na | na | na | na | na | na | | |
| 2016007 | C. belfanti | negative | na | no | 10.9 | prophage | 53.7% | 52.0% | incomplete | 10 | 16 | 322 | 11 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_43041928 | na | na | na | na | na | na | na | na | na | | |
| 2016007 | C. belfanti | negative | na | no | 7.2 | prophage | 53.7% | 58.1% | incomplete | 20 | 9 | 17.171 | 24.403 | PHAGE_Synech_ACG_20141_NC_036927(2) | https://phaster.ca/biocommons/22_43041928 | na | na | na | na | na | na | na | na | na | | |
| 2016007 | C. belfanti | negative | na | no | 3.5 | prophage | 53.7% | 51.6% | questionable | 70 | 9 | 11.015 | 14.546 | PHAGE_Bacter_Diva_NC_028788(1) | https://phaster.ca/biocommons/22_43041928 | na | na | na | na | na | na | na | na | na | | |
| 2017002 | C. belfanti | negative | na | no | 6.8 | prophage | 53.9% | 54.8% | incomplete | 10 | 8 | 31.989 | 38.831 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2017003 | C. belfanti | negative | na | no | 10.9 | prophage | 53.9% | 56.0% | incomplete | 10 | 17 | 54.765 | 65.729 | PHAGE_Mycobacterium_Drbyer_NC_022091(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2017004 | C. belfanti | negative | na | no | 5.1 | prophage | 53.9% | 54.0% | incomplete | 10 | 7 | 1 | 5.179 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2017008 | C. belfanti | negative | na | no | 10.2 | prophage | 53.7% | 54.1% | incomplete | 20 | 16 | 402 | 10.631 | PHAGE_Coryne_Foushouh_NC_042139(9) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018032 | C. belfanti | negative | na | no | 10.9 | prophage | 53.8% | 56.0% | incomplete | 10 | 15 | 78.238 | 89.206 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_R1618b79 | na | na | na | na | na | na | na | na | na | | |
| 2018032 | C. belfanti | negative | na | no | 6.8 | prophage | 53.8% | 55.0% | incomplete | 10 | 8 | 28.433 | 35.275 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_R1618b79 | na | na | na | na | na | na | na | na | na | | |
| 2018032 | C. belfanti | negative | na | no | 7.9 | prophage | 53.8% | 54.3% | incomplete | 20 | 10 | 1 | 7.968 | PHAGE_Coryne_Foushouh_NC_042139(7) | https://phaster.ca/biocommons/22_R1618b79 | na | na | na | na | na | na | na | na | na | | |
| 2018045 | C. belfanti | negative | na | no | 18.0 | prophage | 53.7% | 56.3% | incomplete | 60 | 17 | 68.465 | 86.515 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018045 | C. belfanti | negative | na | no | 35.9 | prophage | 53.7% | 52.9% | incomplete | 30 | 19 | 35.973 | PHAGE_Coryne_Foushouh_NC_042139(13) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | | |
| 2018048 | C. belfanti | negative | na | no | 13.7 | prophage | 53.7% | 55.0% | incomplete | 10 | 16 | 9.102 | 22.879 | PHAGE_Vibrio_Ceto_NC_042091(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018048 | C. belfanti | negative | na | no | 19.7 | prophage | 53.7% | 56.4% | questionable | 70 | 20 | 25.890 | 45.608 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018076 | C. belfanti | negative | na | no | 27.3 | prophage | 53.7% | 53.8% | incomplete | 20 | 14 | 23.488 | 43.823 | PHAGE_Coryne_Foushouh_NC_042139(2) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018076 | C. belfanti | negative | na | no | 27.3 | prophage | 53.7% | 54.8% | incomplete | 30 | 36 | 55.630 | 83.002 | PHAGE_Gordon_BriBri_NC_030942(6) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2018077 | C. belfanti | negative | na | no | 15.3 | prophage | 53.7% | 52.5% | incomplete | 10 | 24 | 3 | 15.376 | PHAGE_Gordon_Neyrac_NC_010040(9) | https://phaster.ca/biocommons/22_6a7211b4 | na | na | na | na | na | na | na | na | na | | |
| 2014016 | C. uclerans | positive | positive | no | 6.8 | prophage | 53.3% | 55.0% | incomplete | 10 | 8 | 28.436 | 35.277 | PHAGE_Gordon_Schwaebitter_NC_031255(1) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2014017 | C. uclerans | positive | positive | no | 10.9 | prophage | 53.3% | 56.0% | incomplete | 10 | 15 | 35.872 | 46.837 | PHAGE_Escher_D108_NC_013594(1) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2014018 | C. uclerans | positive | positive | no | 6.3 | prophage | 53.3% | 59.0% | incomplete | 20 | 7 | 1.799 | 10.167 | PHAGE_Gordon_Blueberry_NC_030943(2) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2014019 | C. uclerans | positive | positive | no | 7.3 | prophage | 53.3% | 53.7% | incomplete | 10 | 13 | 1 | 7.308 | PHAGE_Coryne_Foushouh_NC_042139(7) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2016002 | C. uclerans | negative | na | no | 8.6 | prophage | 53.3% | 55.9% | incomplete | 10 | 10 | 57.676 | 61.148 | PHAGE_Thyph_42X_NC_007020(1) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2017009 | C. uclerans | positive | positive | no | 23.8 | prophage | 53.3% | 53.9% | incomplete | 10 | 27 | 2.329 | 30.995 | PHAGE_Clostridium_Clostridium_NC_028993(4) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2017009 | C. uclerans | positive | positive | no | 38.3 | prophage | 53.3% | 53.6% | incomplete | 20 | 33 | 588 | 38.947 | PHAGE_Gordon_Neyrac_NC_010040(9) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2019012 | C. uclerans | positive | positive | no | 10.9 | prophage | 53.4% | 56.7% | incomplete | 60 | 19 | 209.532 | 228.818 | PHAGE_Gordon_GMAI_NC_030902(5) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2019012 | C. uclerans | positive | positive | no | 11.1 | prophage | 53.4% | 54.9% | incomplete | 20 | 17 | 232.087 | 243.192 | PHAGE_Mycobacterium_Chel_004683(1) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | |
| 2019012 | C. uclerans | positive | positive | no | 35.6 | prophage | 53.4% | 54.9% | incomplete | 40 | 42 | 35.873 | PHAGE_Gordon_Neyrac_NC_010040(10) | https://phaster.ca/biocommons/22_5415d094 | na | na | na | na | na | na | na | na | na | | | |
| 2014018 | C. diphtheriae | positive | positive | no | 19.6 | prophage | 53.6% | 56.1% | incomplete | 30 | 19 | 20.720 | 40.416 | PHAGE_Gordon_GMAI_NC_030902(7) | https://phaster.ca/biocommons/22_6262b332 | na | na | na | na | na | na | na | na | na | | |
| 2014019 | C. diphtheriae | positive | positive | no | 15.2 | prophage | 53.6% | 54.7% | incomplete | 30 | 25 | 43.552 | 58.758 | PHAGE_Rhodocyclus_RM378_NC_004735(1) | https://phaster.ca/biocommons/22_6262b332 | na | na | na | na | na | na | na | na | na | | |
| 2014020 | C. diphtheriae | positive | positive | no | 33.6 | prophage | 53.6% | 52.5% | incomplete | 40 | 44 | 219.353 | 253.024 | PHAGE_Coryne_Foushouh_NC_042139(9) | < | | | | | | | | | | | |

